CDK1 Inhibitor RO-3306 enhances BTKi potency in diffuse large B-cell lymphoma by suppressing JAK2/STAT3 signaling

Q. CHEN[1], C LU[1], L XU[1], L WEI[2], C WANG[1] and L YU[1]

1 Department of Hematology, The Affiliated Huaian No.1 People's Hospital of Nanjing Medical University, Huaian, China

2 Department of Pathology, The Affiliated Huaian No.1 People's Hospital of Nanjing Medical University, Huaian, China

INTRODUCTION

Diffuse large B-cell lymphoma (DLBCL), known for its aggressive nature, comprises roughly 30% of all non-Hodgkin's lymphomas[1]. The past two decades have witnessed considerable progress in demystifying the complex and varied nature of DLBCL, characterized by its distinctive pathological morphology, immunophenotypic attributes, clinical manifestations, and prognoses[2]. Advancements in gene expression profiling (GEP) have paved the way for the recognition of unique DLBCL subgroups. These include prominent subtypes like the activated B-cell-like (ABC) and germinal-center B-cell-like (GCB) groups. Bruton's tyrosine kinase (BTK), a pivotal signaling molecule within the B-cell receptor (BCR) pathway, comes into sharp focus, particularly in sustaining the viability of ABC DLBCL cells harboring wild-type CARD11[3]. The clinical promise of the BTK inhibitor (BTKi) ibrutinib, especially for the ABC subtype, has been underscored in recent clinical trials[4]. Despite the promising trajectory of BTK-targeted therapies, it is critical to acknowledge that DLBCL's response to BTKi is not uniform.

AIM

To elucidate the principal genes influencing BTK inhibitor (BTKi) sensitivity in diffuse large B-cell lymphoma (DLBCL) and to delineate the underlying mechanism.

Within this context, our current investigation unveils the pivotal role of CDK1, a key determinant of BTKi sensitivity in DLBCL. Utilizing a blend of bioinformatics and experimental analyses, we illustrate the influence of CDK1 inhibition (via RO-3306) on enhancing BTKi sensitivity, laying a foundational stone for spearheading personalized therapeutic strategies against DLBCL.

METHOD

Utilizing the microarray dataset GSE138126 extracted from the Gene Expression Omnibus (GEO) database, we conducted a comprehensive analysis to identify differentially expressed genes (DEGs) between BTKi-resistant and BTKi-sensitive cells, employing the "limma" tool. Subsequently, a network of 30 hub genes exhibiting significant connectivity was curated, with CDK1 emerging as the predominant gene. The potential impact of targeting CDK1 using its specific inhibitor, RO-3306, was examined in DLBCL through various methods, including CCK-8 and flow cytometry assays. The efficacy of RO-3306 in augmenting BTKi sensitivity in DLBCL was scrutinized both in cellular models and in mouse xenografts. Further, the mechanistic action of RO-3306 was investigated using RNA-seq and substantiated through qRT-PCR and western blot analyses.

RESULTS

- **CDK1 is the key gene related to ibrutinib resistance in DLBCL** A total of 1658 DEGs were finally identified as related to ibrutinib resistance in DLBCL cells, according to the criteria |logFC|>2 and P value <0.05. Among them, 837 were upregulated. The DEGs were ranked by degree of connectivity (**Figure 1**), and CDK1 was selected as the key gene.
- **CDK1 inhibitor (RO-3306) inhibits viability, prompts apoptosis, and enhances the sensitivity of DLBCL cells to ibrutinib** Using CCK-8 assays, we observed a concentration-dependent decrease of cell proliferation upon treatment with RO-3306. Furthermore, our experiments highlighted the potential synergistic relationship between RO-3306 and ibrutinib. Our data revealed that RO-3306 may act as a potent inhibitor of cell proliferation and promoter the apoptosis in DLBCL cells, potentially enhancing the therapeutic efficacy of ibrutinib in the treatment of DLBCL.
- **RO-3306 inhibits tumor growth and enhances the sensitivity to ibrutinib in vivo** A xenograft tumor growth model was established using BALB/c mice as hosts, into which U2932 cells were subcutaneously implanted. Our investigations vividly indicate that RO-3306 functions as a formidable agent in curtailing tumor growth. Moreover, it was observed to amplify the inhibitory effects of ibrutinib synergistically, shedding light on the potential of combinatory therapy for improved treatment outcomes(**Figure 2**).
- **RO-3306 suppresses the activity of the JAK2/STAT3 signaling pathway** To dissect the intricate dynamics of this pathway under the influence of RO-3306, we executed a meticulous RNA-seq study on U2932 cells subjected to various treatments: DMSO, ibrutinib, RO-3306, and a combination of ibrutinib and RO-3306. This approach facilitated the identification of several differentially expressed RNAs, notably pointing towards an association with JAK2/STAT3 pathway activation(**Figure 3**). Western blot and immunofluorescence experiments were performed, and the results showed that Ro-3306 inhibited JAK2/STAT3 signaling, thereby suppressing the expression of NF-κB, and BCL-2; enhancing the expression of BAX; and affecting the survival, apoptosis, and drug sensitivity of DLBCL cells to Ibrutinib.

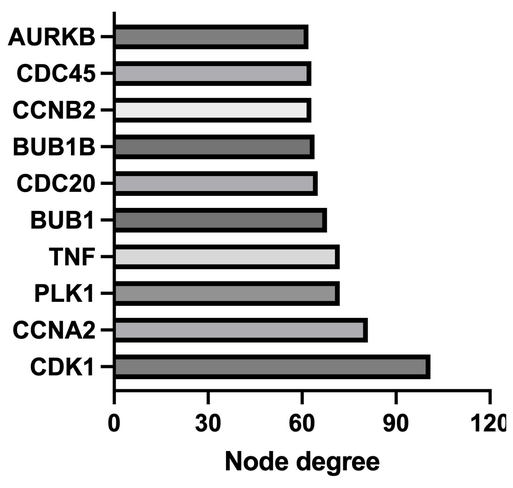


Figure 1 CDK1 was associated with ibrutinib resistance in DLBCL. A set of 10 pivotal genes were identified and arranged based on connectivity significance.

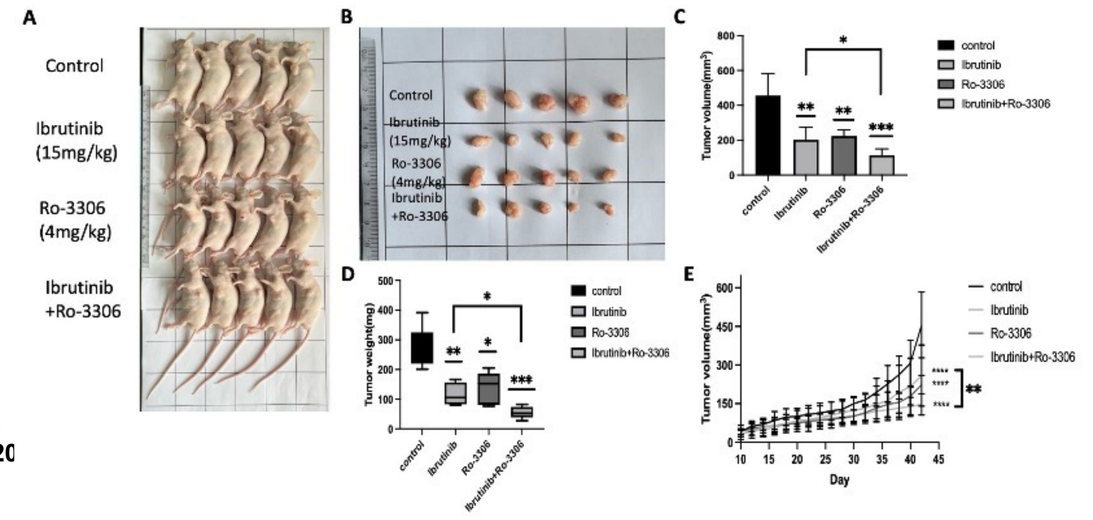


Figure 2 The inhibitory effects of RO-3306 on DLBCL tumor development in mice models. U2932 cells were grafted in BALB/c mice. Post tumor growth to around 100 mm^3, mice were categorized into control and medicated cohorts, each containing four mice. Both Ibrutinib (15 mg/kg) and RO-3306 (4 mg/kg) were given daily. By day 20, resultant tumors were visualized (A, B) and assessed for volume (C) and weight (D). (E) Bi-daily tumor size measurements.

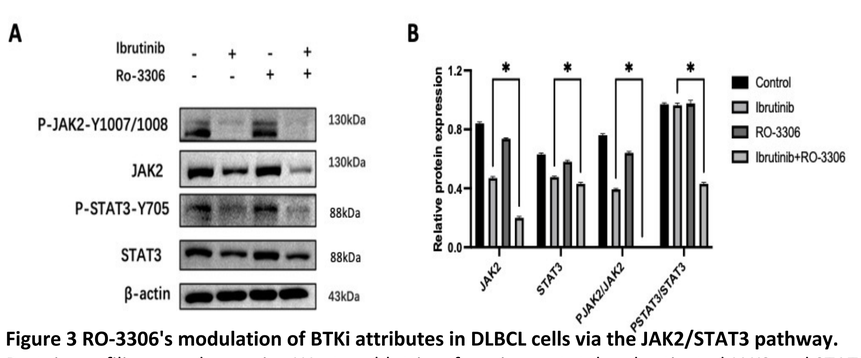


Figure 3 RO-3306's modulation of BTKi attributes in DLBCL cells via the JAK2/STAT3 pathway. Protein profiling was done using Western blotting, focusing on total and activated JAK2 and STAT3 (A). Subsequent calculation of associated protein expression was conducted (B).

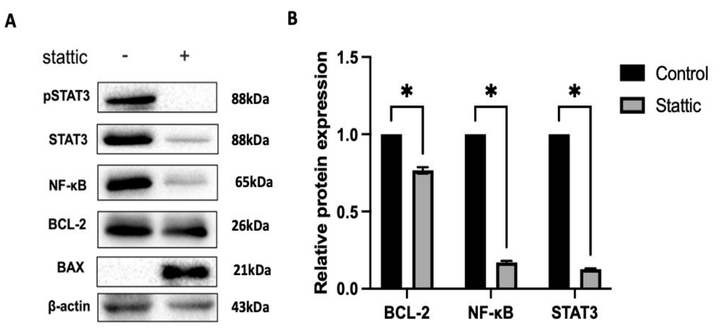
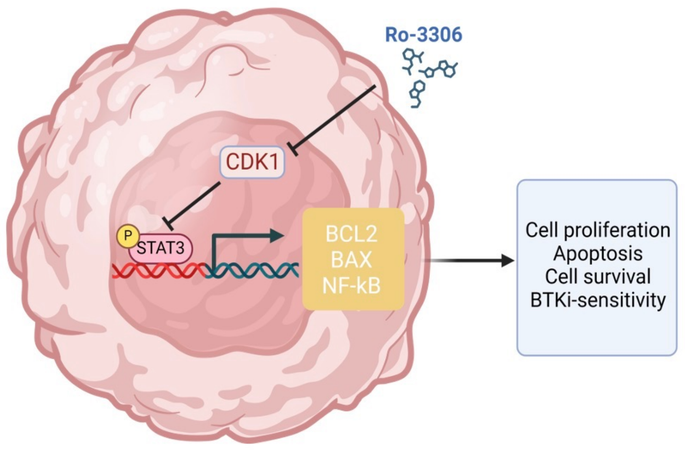


Figure 4 Detection of STAT3 downstream target protein by WB. The results showed that Ro-3306 suppressed the expression of NF-κB, and BCL-2; enhanced the expression of BAX.

CONCLUSIONS

This study pioneers in unveiling pivotal insights into the mechanisms governing BTKi sensitivity in DLBCL, potentially heralding new avenues for targeted therapeutic strategies.



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CONTACT INFORMATION

Correspondence: **Chunling Wang** and **Liang Yu**

Email address:

tomatodrchen@outlook.com (Q. Chen); luchuanyangdyx@163.com (C. Lu); xulei8725@126.com (L. Xu); hayywll@njmu.edu.cn (L. Wei); wcl6506@163 (C. Wang); yuliangha@163.com (L. Yu)